Development of human ES cell lines as a model system for Alzheimer disease drug discovery

Grant Award Details

Development of human ES cell lines as a model system for Alzheimer disease drug discovery

Grant Type: SEED Grant

Grant Number: RS1-00247

Investigator:

Name:	Frank LaFerla
Institution:	University of California, Irvine
Type:	PI

Disease Focus: Alzheimer's Disease, Neurological Disorders

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: Embryonic Stem Cell

Award Value: $473,963

Status: Closed

Progress Reports

Reporting Period: Year 2

View Report

Reporting Period: NCE

View Report

Grant Application Details

Application Title: Development of human ES cell lines as a model system for Alzheimer disease drug discovery

Public Abstract:

Alzheimer disease (AD) is a progressive neurodegenerative disorder that currently affects over 4.5 million Americans. By the middle of the century, the prevalence of AD in the USA is projected to almost quadruple. As current therapies do not abate the underlying disease process, it is very likely that AD will continue to be a clinical, social, and economic burden. Progress has been made in our understanding of AD pathogenesis by studying transgenic mouse models of the disease and by utilizing primary neuronal cell cultures derived from rodents. However, key proteins that are critical to the pathogenesis of this disease exhibit many species-specific differences at both a biophysical and functional level. Additional species differences in other as yet unidentified AD-related proteins are likely to also exist. Thus, there is an urgent need to develop novel models of AD that recapitulate the complex array of human proteins involved in this disease. Cell culture-based models that allow for rapid high-throughput screening and the identification of novel compounds and drug targets are also critically needed. To that end we propose to model both sporadic and familial forms of AD by generating two novel human embryonic stem cell lines (hES cells). Differentiation of these lines along a neuronal lineage will provide researchers with an easily accessible and reproducible neuronal cell culture model of AD. These cells will also allow high-throughput screening and experimentation in neuronal cells with a species-relevant complement of human proteins. In Aim 1 we will develop and characterize hES cell lines designed to model both sporadic and familial forms of AD. To model sporadic AD we will stably transfect HUES7 hES cells (developed by Douglas Melton) with lentiviral constructs coding for human wild type amyloid precursor protein (APP-695) under control of the human APP promoter. APP is well expressed within hES cells and upregulated upon neuronal differentiation. To model familial AD and generate cells that exhibit a more aggressive formation of oligomeric Aï¢ species we will also develop a second hES cell line stably transfected with human APP that includes the Arctic (E693G) mutation.In Aim 2 we will utilize our wild-type APP hES cells to perform a high-throughput siRNA screen. We will utilize AMAXA reverse-nucleofection in conjunction with a human druggable genome siRNA array (Dharmacon) that targets 7309 genes considered to be potential therapeutic targets. Following transfection conditioned media will be examined by a sensitive ELISA to identify novel targets that modulate Aï¢ levels. In addition a Thioflavin S assay will determine any effects on Aï¢ aggregation. Follow-up experiments will confirm promising candidates identified in the high-throughput screen. Taken together these studies aim to establish novel AD-specific hES cell lines and identify promising new therapeutic targets for this devastating disease.

Statement of Benefit to California:

Alzheimer disease (AD) is a progressive neurodegenerative disorder that currently affects over 500 thousand Californians. As the baby-boomer generation ages the prevalence of AD in California is projected to almost quadruple such that 1 in every 45 individuals will be afflicted. As current therapies do not abate the underlying disease process, it is very likely that AD will continue to be a major clinical, social, and economic burden. Some estimates have even suggested that AD alone may bankrupt the current Californian health care system. Progress has been made in our understanding of AD by studying rodent-based models of the disease. However, key proteins that are critical to the disease exhibit many species-specific differences at both a biophysical and functional level. Thus, there is an urgent need to develop novel models of AD that exhibit the complex array of human proteins involved in this disease. Cell culture-based models that also allow for rapid high-throughput screening and the identification of novel compounds and drug targets are also in critical need. The proposed studies aim to utilize human embryonic stem (hES) cells to establish a novel cell culture based model of Alzheimer's disease. Once developed these cells will provide Californian researchers with a unique tool to investigate genes and proteins that influence the progress of AD. In this proposal we will also utilize these hES cells to perform a high-throughput screen of over 7300 genes to identify multiple novel drug targets that may critically regulate the development of this disease. Taken together these studies aim to establish novel AD-specific hES cell lines that can be utilized by multiple Californian researchers to identify promising new therapeutic targets for this devastating disease.